

WHAT IS CLAIMED IS :

- Inv CB7*
- C 50*
- GD*
- J*
- Sub C1*
- o*
- Sub C1*
1. A protein possessing a specific urate oxidase activity of at least 16 U/mg and having the following sequence : *(SEQUENCE ID NO.1)*
Ser Ala Val Lys Ala Ala Arg Tyr Gly Lys Asp Asn Val Arg Val Tyr Lys Val His Lys Asp Glu Lys Thr Gly Val Gln Thr Val Tyr Glu Met Thr Val Cys Val Leu Leu Glu Gly Glu Ile Glu Thr Ser Tyr Thr Lys Ala Asp Asn Ser Val Ile Val Ala Thr Asp Ser Ile Lys Asn Thr Ile Tyr Ile Thr Ala Lys Gln Asn Pro Val Thr Pro Pro Glu Leu Phe Gly Ser Ile Leu Gly Thr 10 His Phe Ile Glu Lys Tyr Asn His Ile His Ala Ala His Val Asn Ile Val Cys His Arg Trp Thr Arg Met Asp Ile Asp Gly Lys Pro His Pro His Ser Phe Ile Arg Asp Ser Glu Glu Lys Arg Asn Val Gln Val Asp Val Val Glu Gly Lys Gly Ile Asp Ile Lys Ser Ser Leu Ser Gly Leu Thr Val Leu Lys Ser Thr Asn Ser Gln Phe Trp Gly Phe Leu Arg Asp Glu Tyr Thr Thr Leu 15 Lys Glu Thr Trp Asp Arg Ile Leu Ser Thr Asp Val Asp Ala Thr Trp Gln Trp Lys Asn Phe Ser Gly Leu Gln Glu Val Arg Ser His Val Pro Lys Phe Asp Ala Thr Trp Ala Thr Ala Arg Glu Val Thr Leu Lys Thr Phe Ala Glu Asp Asn Ser Ala Ser Val Gln Ala Thr Met Tyr Lys Met Ala Glu Gln Ile Leu Ala Arg Gln Gln Leu Ile Glu Thr Val Glu Tyr Ser Leu Pro Asn Lys 20 His Tyr Phe Glu Ile Asp Leu Ser Trp His Lys Gly Leu Gln Asn Thr Gly Lys Asn Ala Glu Val Phe Ala Pro Gln Ser Asp Pro Asn Gly Leu Ile Lys Cys Thr Val Gly Arg Ser Ser Leu Lys Ser Lys Leu preceded if appropriate, by a methionine, or having a substantial degree of homology with that sequence.
- 25 2. A protein according to claim 1, possessing a specific urate oxidase activity of at least 30 U/mg.
- 30 3. A protein according to claim 1 or 2, which presents, by analysis on a bidimensional gel, a spot of molecular mass of about 33.5 kDa and an isoelectric point around 8.0, representing at least 90 % of the protein mass.
- 35 4. A protein according to anyone of claims 1 to 3, having a purity degree, determined by liquid chromatography on a C8 grafted silica column, higher than 80 %.
5. A protein according to anyone of claims 1 to 4, having an isoelectric point around 8.0.

- ~~C~~ ~~Claim 2~~
6. A protein according to anyone of claims 1 to 4, which carries a blocking group on the amino-terminal serine having preferably a molecular mass around 43 units of atomic mass.
7. A drug containing a protein according to anyone of claims 05 1 to 6.

8. A recombinant gene which has the DNA sequence coding for the protein having the following sequence : *(SEQUENCE ID NO. 2)*

Met Ser Ala Val Lys Ala Ala Arg Tyr Gly Lys Asp Asn Val Arg Val
10 Tyr Lys Val His Lys Asp Glu Lys Thr Gly Val Gln Thr Val Tyr Glu
Met Thr Val Cys Val Leu Leu Glu Gly Glu Ile Glu Thr Ser Tyr Thr Lys
Ala Asp Asn Ser Val Ile Val Ala Thr Asp Ser Ile Lys Asn Thr Ile Tyr
Ile Thr Ala Lys Gln Asn Pro Val Thr Pro Pro Glu Leu Phe Gly Ser Ile
Leu Gly Thr His Phe Ile Glu Lys Tyr Asn His Ile His Ala Ala His Val
15 Asn Ile Val Cys His Arg Trp Thr Arg Met Asp Ile Asp Gly Lys Pro
His Pro His Ser Phe Ile Arg Asp Ser Glu Glu Lys Arg Asn Val Gln
Val Asp Val Va Glu Gly Lys Gly Ile Asp Ile Lys Ser Ser Leu Ser Gly
Leu Thr Val Leu Lys Ser Thr Asn Ser Gln Phe Trp Gly Phe Leu Arg Asp
Glu Tyr Thr Thr Leu Lys Glu Thr Trp Asp Arg Ile Leu Ser Thr Asp Val
20 Asp Ala Thr Trp Gln Trp Lys Asp Phe Ser Gly Leu Gln Glu Val Arg Ser
His Val Pro Lys Phe Asp Ala Thr Trp Ala Thr Ala Arg Glu Val Thr Leu
Lys Thr Phe Ala Glu Asp Asn Ser Ala Ser Val Gln Ala Thr Met Tyr Lys
Met Ala Glu Gln Ile Leu Ala Arg Gln Gln Leu Ile Glu Thr Val Glu Tyr
Ser Leu Pro Asn Lys His Tyr Phe Glu Ile Asp Leu Ser Trp His Lys Gly
25 Leu Gln Asn Thr Gly Lys Asn Ala Glu Val Phe Ala Pro Gln Ser Asp Pro
Asn Gly Leu Ile Lys Cys Thr Val Gly Arg Ser Ser Leu Lys Ser Lys Leu

9. A recombinant gene according to claim 8, which permits the expression in the prokaryotic microorganisms.

30 10. A recombinant gene according to claim 9, wherein the DNA sequence contains the following sequence *(SEQUENCE ID NO:3)*:

ATGTGTGCGG TAAAAGCAGC GCGCTACGGC AAGGACAATG TTCCGCTCA
CAAGGTTAAC AAGGACGAGA AGACCGGTGT CCAGACGGTG TACGAGATGA
CCGTCTGTGT GCTTCTGGAG GGTGAGATTG AGACCTCTTA CACCAAGGCC
GACAACAGCG TCATTGTCGC AACCGACTCC ATTAAGAACAA CCATTTACAT
05 CACCGCCAAG CAGAACCCCCG TTACTCCTCC CGAGCTGTTC GGCTCCATCC
TGGGCACACA CTTCATTGAG AAGTACAACC ACATCCATGC CGCTCACGTC
AACATTGTCT GCCACCGCTG GACCCGGATG GACATTGACG GCAAGCCACA
CCCTCACTCC TTCATCCGCG ACAGCGAGGA GAAGCGGAAT GTGCAGGTGG
ACGTGGTCGA GGGCAAGGGC ATCGATATCA AGTCGTCTCT GTCCGGCTG
10 ACCGTGCTGA AGAGCACCAA CTCGCAGTTC TGGGGCTTCC TGCGTGACGA
GTACACCACA CTTAAGGAGA CCTGGGACCG TATCCTGAGC ACCGACGTCG
ATGCCACTTG GCAGTGGAAAG AATTTCAGTG GACTCCAGGA GGTCCGGTCG
CACGTGCCTA AGTTCGATGC TACCTGGGCC ACTGCTCGCG AGGTCACTCT
GAAGACTTTT GCTGAAGATA ACAGTGCCAG CGTGCAGGCC ACTATGTACA
15 AGATGGCAGA GCAAATCCTG GCGCGCCAGC AGCTGATCGA GACTGTCGAG
TACTCGTTGC CTAACAAGCA CTATTCGAA ATCGAISCTGA GCTGGCACAA
GGGCCTCCAA AACACCGGCA AGAACGCCGA GGTCTTCGCT CCTCAGTCGG
ACCCCAACGG TCTGATCAAG TGTACCGTCG GCCGGTCCCTC TCTGAAGTCT
AAATTG.

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11. A recombinant gene according to claim 8, which permits the expression in the eukaryotic cells.

12. A recombinant gene according to claim 11, wherein the DNA sequence contains the following sequence : *(SEQUENCE ID NO: 1)*

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ATGTCTGCTG TTAAGGCTGC TAGATACGGT AAGGACAACG TTAGACTCTA
CAAGGTTCAC AAGGACGAGA AGACCGGTGT CCAGACGGTG TACGAGATGA
CCGTCTGTGT GCTTCTGGAG GGTGAGATTG AGACCTCTTA CACCAAGGCC
GACAACAGCG TCATTGTCGC AACCGACTCC ATTAAGAACAA CCATTACAT
05 CACCGCCAAG CAGAACCCCCG TTACTCCTCC CGAGCTGTTG GGCTCCATCC
TGGGCACACCA CTTCATTGAG AAGTACAACC ACATCCATGC CGCTCACGTC
AACATTGCTC GCCACCGCTG GACCCGGATG GACATTGACG GCAAGCCACA
CCCTCACTCC TTCATCCGCG ACAGCGAGGA GAAGCGGAAT GTGCAGGTGG
ACGTGGTCGA GGGCAAGGCC ATCGATATCA AGTCGTCTCT GTCCGGCCTG
10 ACCGTGCTGA AGAGCACCAA CTCGCAGTTC TGGGGCTTCC TGCGTGACGA
GTACACCACA CTTAAGGAGA CCTGGGACCG TATCCTGAGC ACCGACGTGCG
ATGCCACTTG GCAGTGGAAAG AAATCGATG GACTCCAGGA GGTCCGCTCG
CACGTGCCTA AGTTCGATGC TACCTGGGCC ACTGCTCGCG AGGTCACTCT
GAAGACTTTT GCTGAAGATA ACAGTGCAG CGTGCAGGCC ACTATGTACA
15 AGATGGCAGA GCAAATCCTG GCGCGCCAGC AGCTGATCGA GACTGTCGAG
TACTCGTTGC CTAACAAGCA CTATTCGAA ATCGACCTGA GCTGGCACAA
GGGCCTCCAA AACACCGGCA AGAACGCCGA GGTCTTCGCT CCTCAGTCGG
ACCCCAACGG TCTGATCAAG TGTACCGTCG GCCGGTCCCTC TCTGAAGTCT
AAATTG.

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13. A recombinant gene according to claim 8, which permits the expression in the animal cells.

14. A recombinant gene according to claim 13, wherein the DNA sequence contains the following sequence : A (SEQUENCE ID NO. 6)

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	-ATGTC	CGCAGTAAAA	GCAGCCCCT	ACGGCAAGGA
	CAATGTCCGC	GTCTACAAGG	TTCACAAGGA	CGAGAAGACC
	CGGTGTACGA	GATGACCGTC	TGTGTGCTTC	TGGAGGGTGA
	TCTTACACCA	AGGCCGACAA	CAGCGTCATT	GTCGCAACCG
05	GAACACCATT	TACATCACCG	CCAAGCAGAA	CCCCGTTACT
	TGTTGGCTC	CATCCTGGGC	ACACACTTCA	TTGAGAAAGTA
	CATGCCGCTC	ACGTCAACAT	TGTCTGCCAC	CGCTGGACCC
	TGACGGCAAG	CCACACCCCTC	ACTCCTTCAT	CCGCGACAGC
	GGAATGTGCA	GGTGGACGTG	GTGAGGGCA	AGGGCATCGA
10	TCTCTGTCCG	GCCTGACCGT	GCTGAAGAGC	ACCAAACTCGC
	CTTCTCGGT	GACGAGTACA	CCACACTTAA	GGAGACCTGG
	TGAGCACCAGA	CGTCGATGCC	ACTTGCGAGT	GGAAGAATT
	CAGGAGGTCC	GCTCGCACGT	GCCTAACATT	GATGCTACCT
	TCGCGAGGTC	ACTCTGAAGA	CTTGCTGA	AGATAACAGT
15	AGGCCACTAT	GTACAAGATG	GCAGAGCAA	TCCTGGCGCG
	ATCGAGACTG	TCGAGTACTC	GTGCTAAC	CCAGCAGCTG
	CCTGAGCTGG	CACAAGGGCC	TCCAAAACAC	AAGCACTATT
	TCGCTCCTCA	GTCGGACCCC	AACGGTCTGA	TCAGTGTAC
	TCCTCTCTGA	AGTCTAAATT	G	CGTCGGCCGG

20 preceded by a non-translated 5' sequence favoring expression in animal cells,

15. Recombinant gene according to claim 14, wherein the
 non-translated 5' sequence favoring expression in animal cells
 comprises the sequence ~~(SEQUENCE ID NO: 5)~~ located immediately upstream
 from the sequence described in claim 14.

25 16. An expression vector carrying a recombinant gene
 according to any one of claims 8 to 15 with the means necessary for
 its expression.

17. An expression vector according to claim 16, which carries
 at least one selection marker.

30 18. An expression vector according to claim 17, which has the
 characteristics of one of plasmids pEMR469, pEMR473, and pEMR 515.

19. Prokaryotic microorganisms which are transformed by an
 expression vector according to claim 16, carrying a recombinant
 gene according to claim 9.

20. Eukaryotic cells which are transformed by one of the expression vectors according to any one of claims 16 to 18, carrying the recombinant gene according to claim 11.
21. A strain of Saccharomyces cerevisiae which is transformed
05 by one of the expression vectors according to any one of claims 16 to 18.
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22. A strain according to claim 21, which carries a mutation on at least one of the genes responsible for the synthesis of leucine or uracil.
- 10 23. A strain according to claim 22, which carries a mutation on at least one of the LEU2 and URA3 genes.
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- Subst 4*
24. A process for producing a recombinant urate oxidase which comprises the steps of :
- 15 1) cultivating a strain according to claims 21 to 23 ;
2) lysing the cells ;
3) isolating and purifying the recombinant urate oxidase contained in the lysate.
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- 11c5*
25. Animal cells containing a recombinant gene according to claim 13 with the means necessary for its expression.
- 20 26. Animal cells containing an expression vector according to claim 16, carrying a recombinant gene according to claim 14.
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